Amendments to the Specification

Please amend the specification as follows:

On page 1, line 1, please substitute the following title:

~~VACCINE~~OUTER MEMBRANE VESICLES AND USES THEREOF

On page 7, beginning on line 20, please substitute the following paragraph:

The kanamycin-resistance cassette (KAN) replaces *msbA* in the mutant, leaving only 131 bp at the 3' end (M). Primers used for the disruption procedure and cloning of *msbA* are indicated with arrows. Primer sequences are (A) CCCAAAGCGAAGTGGTCGAA (SEQ ID NO: 6); (B) GTCGACTATCGGTAGGGCGGGAACTG (*Acc*I restriction site is underlined) (SEQ ID NO: 7); (C) GTCGACGACCGCATCATCGTGATGGA (*Acc*I restriction site is underlined) (SEQ ID NO: 8); (D) TTCGTCGCTGCCGACCTGTT (SEQ ID NO: 9); (E) TTCATATGATAGAAAAACTGACTTTCGG (*Nde*I restriction site is underlined) (SEQ ID NO: 10); (F) GACGTCCCATTTCGGACGGCATTTTGT (*Aat*II restriction site is underlined) (SEQ ID NO: 11). Predicted promoter (P) and terminator (T) sequences are indicated. ORFs indicated with NMB1918 and NMB1920 putatively code for a malonyl CoA-acyl carrier protein transacylase and GMP synthase, respectively.

On page 14, beginning on line 4, please substitute the following paragraph:

Further Neisserial OMP loops that may be substituted for Imp loops (particularly loops 3 and/or 8) are PorA loop 4 [or variable region 2] ~~(see http//neisseria.org/nm/typing/porA/)~~; PorA loop 5 (described in "Topology of outer membrane porins in pathogenic Neisseria spp", van der Ley, Poolman, etc.., Infect Immun 1991, 59, 2963-71; [[ ]]its sequence in PorA P1.7,16 (H44/76) loop 5 being: RHANVGRNAFELFLIGSGSDQAKGTDPLKNH, SEQ ID NO: 12); LbpA surface exposed loops 4, 5, 7, 10 and 12, corresponding to amino acids 210-342, 366-441, 542-600, 726-766 and 844-871, respectively, with 12 being preferred (sequence KGKNPDELAYLAGDQKRYSTKRASSSWST), SEQ ID NO: 13) [see Prinz et al.

1999 J Bacter. 181:4417 for further details on LbpA surface loops incorporated by reference herein]; NspA surface exposed loops 1, 2, 3 or 4, corresponding to amino acid sequence 25-54, 61-87, 103-129 and 149-164, respectively, preferably where loop 2 (e.g. FAVDYTRYKNYKAPSTDFKLYSIGASA, SEQ ID NO: 14) and/or 3 (e.g. ARLSLNRASVDLGGSDSFSQTSIGLGVL, SEQ ID NO: 15) is inserted (as these loops are quite small not all the Imp loop 2 and/or 8 would be ideally removed to introduce these loops, and if both are to be introduced, it is preferred that they are introduced on loop 2 or 8 (or vice versa) in order to try to preserve the conformational epitope that exists between loops 2 and 3 of NspA) [see Vandeputte-Rutten et al 2003 JBC 278:24825 for more details on NspA loops, incorporated by reference herein]; any of the surface exposed loops of Omp85 (see Science 2003 299:262-5, and supporting online material Fig S4, incorporated by reference herein).

On page 30, beginning on line 16, please substitute the following paragraphs:

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and ~~Tween 80~~ TWEEN® 80 (polysorbate 80). In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% ~~tween 80~~ TWEEN® 80 (polysorbate 80). Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. ~~Tween 80~~ TWEEN® 80 (polysorbate 80), in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

On page 46, beginning on line 30, please substitute the following paragraph:

Neisseria meningitidis encodes two RTX proteins, referred to as FrpA & FrpC secreted upon iron limitation (Thompson *et al.*, (1993) J. Bacteriol. 175:811-818; Thompson *et al.*, (1993) Infect. Immun. 61:2906-2911). The RTX (Repeat ToXin) protein family [[ ]]have in common a series of 9 amino acid repeat near their C-termini with the consensus: Leu Xaa Gly Gly Xaa Gly (Asn/Asp) Asp Xaa, SEQ ID NO: 16 / SEQ ID NO: 30. ($LXGGXGN_{/D}DX$). The repeats in *E. coli* HlyA are thought to be the site of Ca2+ binding. As represented in Figure 4, meningococcal FrpA and FrpC proteins, as characterized in strain FAM20, share extensive amino-acid similarity in their central and C-terminal regions but very limited similarity (if any) at the N-terminus. Moreover, the region conserved between FrpA and FrpC exhibit some polymorphism due to repetition (13 times in FrpA and 43 times in FrpC) of a 9 amino acid motif.

On page 48, beginning on line 21, please substitute the following paragraph:

This process can advantageously enhance the stability and/or immunogenicity (providing T-cell help) and/or antigenicity of the LOS antigen within the bleb formulation – thus giving T-cell help for the T-independent oligosaccharide immunogen in its most protective conformation – as LOS in its natural environment on the surface of meningococcal outer membrane. In addition, conjugation of the LOS within the bleb can result in a detoxification of the LOS (the Lipid A portion being stably buried in the outer membrane thus being less available to cause toxicity). Thus the detoxification methods mentioned herein of isolating blebs from $htrB^-$ or $msbB^-$ mutants, or by adding non toxic peptide functional equivalent of polymyxin B [a molecule with high affinity to Lipid A] to the composition (see WO 93/14115, WO 95/03327, Velucchi et al (1997) J Endotoxin Res 4: 1-12, and EP 976402 for further details of non-toxic peptide functional equivalents of polymyxin B – particularly the

use of the peptide SAEP 2 (of sequence KTKCKFLKKC, SEQ ID NO: 17, where the 2 cysteines form a disulphide bridge)) may not be required (but which may be added in combination for additional security). Thus the inventors have found that a composition comprising blebs wherein LOS present in the blebs has been conjugated in an intra-bleb fashion to outer membrane proteins also present in the bleb can form the basis of a vaccine for the treatment or prevention of diseases caused by the organism from which the blebs have been derived, wherein such vaccine is substantially non-toxic and is capable of inducing a T-dependent bactericidal response against LOS in its native environment.

On page 61, beginning on line 29, please substitute the following Table 1:

Table 1. Oligonucleotides (primers) used in this study. Underlined sequences indicate restriction sites: *Acc*I in primers B, C, E and F; *N*deI in primers G and H, *Aat*II in primer D and *Bam*HI in primer I. Dashed line in primer F indicates the Neisserial DNA uptake sequence.

	Sequence (5'-3')	Purpose
A	ATGCCTGCAACCTTCAAGTG, SEQ ID NO: 18	5' primer for cloning of NMB0279
B	ATGTCGACAATCGCCCCTCAAGTCGGTTTG, SEQ ID NO: 19	3' primer for cloning of NMB0279
C	ATGTCGACTACCTGCGGCCGGATTATGC, SEQ ID NO: 20	5' primer for cloning of 3' end of imp
D	ATGACGTCTCAGGGTCGTTTGTTGCGTCCGGC, SEQ ID NO: 21	3' primer for cloning of 3' end of imp
E	AGCGTCGACTTCAGACGGCCACGTTGTGTC, SEQ ID NO: 22	5' primer for cloning of Kan-cassette
F	AGCGTCGACGCTGAGGTCTGCCTCGTG, SEQ ID NO: 23	3' primer for cloning of Kan-cassette
G	ATCATATGGCTCGTTTATTTTCACTCAA	5' primer for cloning of

	ACC, SEQ ID NO: 24	complete imp gene into pEN11
H	TGCATATGGATGCCGTTGCGGCGGAG, SEQ ID NO: 25	5' primer for cloning of imp into pET11a
I	TGGGATCCTCAGGGTCGTTTGTTGCGTC C, SEQ ID NO: 26	3' primer for cloning of imp into pET11a